

APPLICANTS: Polakiewicz *et al.*
U.S.S.N.: 10/694,874

Listing of Claims

1. (Canceled)
2. (Canceled)
3. (Canceled)
4. (Canceled)
5. (Canceled)
6. (Withdrawn) A method for detecting phosphorylated IRS-1 and/or phosphorylated IRS-2 in a biological sample, said method comprising the steps of:
 - (a) contacting a biological sample potentially, or suspected of, containing phosphorylated IRS-1 and/or phosphorylated IRS-2 with at least one antibody of claim 1, under conditions suitable for formation of an antibody-IRS complex; and
 - (b) detecting the presence of said complex in said sample, wherein the presence of said complex indicates the presence of phosphorylated IRS-1 (Ser1101) and/or phosphorylated IRS-2 (Ser1149) in said sample.
7. (Withdrawn) The method of claim 6, wherein said biological sample is obtained from a subject at risk of, or suspected of, having type 2 diabetes.
8. (Withdrawn) The method of claim 6, wherein said biological sample has been contacted with at least one Protein Kinase C (PKC) inhibitor or PKC theta inhibitor, or is obtained from a subject treated with such inhibitor.
9. (Withdrawn) The method of claim 6, wherein said biological sample has been contacted with a compound being tested for inhibition of PKC activity or expression.

APPLICANTS: Polakiewicz *et al*
U.S.S.N.: 10/694,874

10. (Canceled)

11. (Withdrawn) A method for detecting PKC theta activity in a biological sample, said method comprising the steps of:

- (a) contacting said biological sample with at least one antibody of claim 1 under conditions suitable for formation of an antibody-IRS complex;
- (b) detecting the presence of said complex in said biological sample, wherein the presence of said complex indicates the presence of phosphorylated IRS-1 (Ser1101) and/or phosphorylated IRS-2 (Ser1149) in said test tissue.

12. (Withdrawn) The method of claim 11, further comprising the step (c) comparing the level of complex detected in step (b) with the level of complex in a control sample with known PKC theta activity, wherein a difference in IRS-1 (Ser1101) and/or IRS-2 (Ser1149) phosphorylation levels between said biological sample and said control sample indicates altered PKC theta activity in said biological sample.

13. (Withdrawn) The method of claim 11, wherein said biological sample is obtained from a subject at risk of, or suspected of, having type 2 diabetes.

14. (Withdrawn) The method of claim 11, wherein said biological sample has been contacted with at least one PKC inhibitor or PKC theta inhibitor, or is obtained from a subject treated with such inhibitor.

15. (Withdrawn) The method of claim 11, wherein said biological sample has been contacted with a compound being tested for inhibition or PKC activity or expression.

16. (Canceled)

17. (new) An isolated antibody that binds to human Insulin Receptor Substrate-1 (IRS-1) when phosphorylated at serine 1101 (SEQ ID NO: 1), but does not bind human IRS-1 when not phosphorylated at serine 1101.

APPLICANTS: Polakiewicz *et al.*
U.S.S.N.: 10/694,874

18. (new) The antibody of claim 17, wherein said antibody further binds to human Insulin Receptor Substrate-2 (IRS-2) when phosphorylated at serine 1149 (SEQ ID NO: 2) , but does not bind human IRS-2 when not phosphorylated at serine 1149.
- 19 (new) The antibody of claim 17, wherein said antibody further binds to murine IRS-1 when phosphorylated at serine 1095 (SEQ ID NO: 3), but does not bind murine IRS-1 when not phosphorylated at serine 1095.
20. (new) The antibody of claim 17, wherein said antibody further binds to murine IRS-2 when phosphorylated at serine 1138 (SEQ ID NO: 4), but does not bind murine IRS-2 when not phosphorylated at serine 1138.
21. (new) An isolated antibody that binds to human IRS-2 when phosphorylated at serine 1149, but does not bind human IRS-2 when not phosphorylated at serine 1149.
22. (new) The antibody of claim 21, wherein said antibody further binds to human IRS-1 when phosphorylated at serine 1101, but does not bind human IRS-1 when not phosphorylated at serine 1101.
23. (new) The antibody of claim 21, wherein said antibody further binds to murine IRS-1 when phosphorylated at serine 1095, but does not bind murine IRS-1 when not phosphorylated at serine 1095.
24. (new) The antibody of claim 21 wherein, said antibody further binds to murine IRS-2 when phosphorylated at serine 1138, but does not bind murine IRS-2 when not phosphorylated at serine 1138.
25. (new) An isolated antibody that binds to murine IRS-1 when phosphorylated at serine 1095, but does not bind murine IRS-1 when not phosphorylated at serine 1095.
26. (new) The antibody of claim 25, wherein said antibody further binds to human IRS-1 when phosphorylated at serine 1101, but does not bind human IRS-1 when not phosphorylated at serine 1101.
27. (new) The antibody of claim 25, wherein said antibody further binds to human IRS-2 when phosphorylated at serine 1149, but does not bind human IRS-2 when not phosphorylated at serine 1149.
28. (new) The antibody of claim 25, wherein said antibody further binds to murine IRS-2

APPLICANTS: Polakiewicz *et al.*
U.S.S.N.: 10/694,874

when phosphorylated at serine 1138, but does not bind murine IRS-2 when not phosphorylated at serine 1138.

29. (new) An isolated antibody that binds to murine IRS-2 when phosphorylated at serine 1138, but does not bind murine IRS-2 when not phosphorylated at serine 1138.

30. (new) The antibody of claim 29, wherein said antibody further binds to human IRS-1 when phosphorylated at serine 1101, but does not bind human IRS-1 when not phosphorylated at serine 1101.

31. (new) The antibody of claim 29, wherein said antibody further binds to human IRS-2 when phosphorylated at serine 1149, but does not bind human IRS-2 when not phosphorylated at serine 1149.

32. (new) The antibody of claim 29, wherein said antibody further binds to murine IRS-1 when phosphorylated at serine 1095, but does not bind murine IRS-1 when not phosphorylated at serine 1095.

33. (new) The antibody as in one of claims 17, 21, 25 or 29, wherein said antibody is polyclonal.

34. (new) The antibody as in one of claims 17, 21, 25 or 29, wherein said antibody is monoclonal.

35. (new) A hybridoma cell line producing any one of the antibodies of claim 34.

36. (new) A kit for the detection of phosphorylated human IRS-1 (Ser1101) in a biological sample, said kit comprising (a) the antibody of claim 17 and (b) at least one secondary antibody conjugated to a detectable group.

37. (new) A kit for the detection of phosphorylated human IRS-2 (Ser1149) in a biological sample, said kit comprising (a) the antibody of claim 21 and (b) at least one secondary antibody conjugated to a detectable group.

38. (new) A kit for the detection of PKC theta activity in a biological sample, said kit comprising (a) at least one antibody of claims 17, 21, 25 or 29 and (b) at least one secondary antibody conjugated to a detectable group.